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FURTHER TISSUE CULTURE STUDIES OF NON-GONOCOCCAL URETHRITIS AND REITER'S SYNDROME*

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The failure to demonstrate a viral aetiology in non-gonococcal urethritis by means of Hela cell cultures has been reported (Ford, 1956). The study has been continued with cultures of human amnion and human conjunctival cells (Chang, 1954) and the methods and experiments are recorded below although no virus was found.

Materials and Methods

A new series of male patients with urethritis following sexual intercourse was investigated. Cases were selected for study when urethral smears and cultures revealed no gonococci, and a visible purulent or semi-purulent discharge showed, on stained smear, a moderate to large number of pus cells, but no predominant organism in significant numbers. Table I (overleaf) gives the relevant clinical data of the 24 cases.

In the present study the urethra was irrigated with 5 to 10 ml. culture medium, instead of Hanks' solution, so that the washings could be added to the cells directly, or at a 50 per cent. concentration, thus avoiding the 1 in 10 dilution previously used. The tissue culture methods were similar to those employed for the Hela cells, except that the 2 per cent. calf serum medium was prepared with inactivated serum heated to 56° C. for 30 minutes, and that medium ML 192/2 (Waymouth, 1956), in which 0·05 per cent. bovine albumin replaces the serum component, was used for most of the amnion cultures. The Chang conjunctival cells, obtained originally from Microbiological Associates, proved less satisfactory than the Hela strain for prolonged observation, because of a tendency to become rounded and peel off the glass. Moreover, their behaviour in the 2 per cent. calf serum medium was variable; on occasions the cells remained healthy for up to 18 days, whereas at other times non-specific degenerative changes were visible in control cultures after 9 days. Cases 9 to 16 were observed twice for cytopathogenic effects against Chang cells; initially, when fresh washings were used, the control cultures appeared unhealthy within the first 9 days, so that the experiments were abandoned and repeated with stored washings 2 months later, at which time the control cultures remained healthy for 18 days. Human amnion cultures, prepared from fresh placentas by repeated 30 to 45 minute exposures to 0·25 per cent. trypsin, remained in good condition for up to 20 days in ML 192/2 and, if changed to 5 per cent. heated calf serum medium after an initial period in ML 192/2, would allow up to 35 days' observation. Table II gives the experimental procedures adopted for the cases, and indicates the occasions when passage of a questionable cytopathogenic effect was performed.

Results

Table II (p. 55) shows that up to three passage experiments were performed in each of the first eight cases. On these occasions, the inoculated cultures seemed unhealthy as compared with their controls, but only three times was the change marked and in all the non-specific degeneration ultimately failed to appear. When the study was continued with the next eight cases, the control cultures deteriorated spontaneously within a 9-day period, as already described. The re-testing of these specimens and the investigation of the last eight patients were performed 2 months later with stored washings; however, although single passages were done on thirteen occasions, no passageable change could be demonstrated.†

Fresh urethral washings from seventeen cases, in addition to one conjunctival swab and two knee joint fluids, were examined for a cytopathogenic effect in human amnion cells. Altogether, 2 per cent. calf serum medium was used for four experiments, ML 192/2 alone for three, and for the remaining thirteen the cells were maintained in ML 192/2 for a period of 3–20 days, after which 5 per cent. heated

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† Please see Addendum for Case 9.

TABLE I
CLINICAL DATA

Case No.	Age (yrs)	Number of Episodes of Past Urethritis	Number of Episodes of Proved Gonococcal Urethritis	Approximate Interval: Between Exposure and Onset of Discharge (days)	Approximate Interval: Between Onset of Discharge and Urethral Washing (days)	Number of Pus Cells in Smear	Bacterial Content of Smear
1	30	1	0	15	15	+++	? Abacterial
2	20	0	0	24	5*	+++	? Abacterial
3	42	1	1	?†	6	+++	Moderate Number of Gram + cocco-bacilli
4	38	1	1	10	20	++	Scattered Gram + cocco-bacilli
5	26	0	0	6	41	+++	Scattered Gram + cocci and Gram + and − bacilli
6	21	1	1	5	9	++	Scattered Gram + cocci and Gram + and − bacilli
7	39	6	0	7	9	+++	? Abacterial
8	23	0	0	11	6	+++	? Abacterial
9	34	5	0	17	5	+++	? Abacterial
10	29	3	0	?†	16	+++	? Abacterial
11	34	6	2	3	22	+++	Moderate Number of Gram + cocci and bacilli
12	26	0	0	5	3	++	? Abacterial
13	26	1	0	1	5	++	? Abacterial
14	19	0	0	3	4	++	? Abacterial
15	20	0	0	6	11	+++	? Abacterial
16	33	0	0	6	6	+++	? Abacterial
17	31	8	7	10	7	+++	? Abacterial
18	20	1	0	7	9	+++	Moderate Number of Gram + cocco-bacilli
19	37	2	0	?†	14	+++	? Abacterial
20	28	0	0	3	11	+++	? Abacterial
21	23	0	0	21	8	+++	? Abacterial
22	20	0	0	9	12	++	? Abacterial
23	46	3–5	0	7	14	+++	? Abacterial
24	28	0	0	5	5	+++	? Abacterial

* From onset of relapse, 39 days from initial urethritis. † Multiple recent exposures.

calf serum was employed, and the cultures further observed. These procedures are outlined in Table II, which shows that ten amnion cultures were followed for 3 to 5 weeks, and that in seven cases fluid from the original tubes was passed without any significant change developing in the passage cultures.

Patients 15 and 18 had Reiter's syndrome. The former complained of conjunctivitis on the 14th day after the urethral washing was taken, and on the following day he had pain and swelling of the left knee from which, on the 17th day, synovial fluid was aspirated.

In Case 18, conjunctivitis and arthritis of the left knee arose 7 days after the onset of the urethritis, and conjunctival, synovial, and urethral specimens were collected 2 days later.

In addition to these cases, two other patients with arthritis had been investigated in the previous year when Hela cell cultures were being used.

One patient, a man aged 25, developed Reiter's syndrome in November, 1955, and the arthritis persisted for 5 months; after another exposure with the same consort, the urethritis recurred, and the arthritis relapsed. In May, 1956, urethral washings were obtained while he had a purulent discharge, but the absence of synovial effusions prevented the collection of joint exudate. Hela cell cultures failed to show any cytopathogenic change suggestive of a virus, and the presence of a penicillin and streptomycin resistant staphylococcus necessitated the addition of chloramphenicol to the medium.

The second patient, a man aged 22, complained of urethritis and a painful swelling of the right knee early in

TABLE II

EXPERIMENTAL DATA

Case No.	Number of Days Cultures were Observed										
	Chang Conjunctival Cells						Human Amnion Cells				
	Fresh Washings				Stored Washings		Fresh Washings				
	Original Cultures in 2 per cent. Calf Serum (C.S.)	Passage Cultures			Original Cultures	Passage Cultures	Original Cultures				Passage Cultures
		1st	2nd	3rd			In 2 per cent. C.S.	In 192/2	192/2→ 5 per cent. C.S.		
									In 192/2	In 5 per cent. C.S.	
1	14	9	9	12	—	—	—	—	—	—	—
2	18	9	9	12	—	—	—	—	—	—	—
3	10	17	12	—	—	—	—	—	—	—	—
4	18	6	9	15	—	—	—	—	—	—	—
5	6	9	12	11	—	—	—	—	—	—	—
6	12	8	12	—	—	—	—	—	—	—	—
7	3	6	17	12	—	—	—	—	—	—	—
8	14	9	—	—	—	—	13	—	—	—	14
9	9	—	—	—	18	11‡	11	—	—	—	—
10	9	—	—	—	18	—	17	—	—	—	—
11	9	—	—	—	18	—	14	—	—	—	—
12	6	—	—	—	18	—	—	12	—	—	—
13	9	—	—	—	18	—	—	9	—	—	—
14	9	—	—	—	18	—	—	9	—	—	—
15	5	—	—	—	18	18	—	—	19	16	26
15K*	—	—	—	—	18	15	—	—	4	27	19
16	5	—	—	—	18	18	—	—	19	9	—
17	—	—	—	—	18	18	—	—	4	12	—
18	—	—	—	—	18	18	—	—	20	9	26
18K*	—	—	—	—	18	18	—	—	4	26	—
18E†	—	—	—	—	—	—	—	—	4	17	—
19	—	—	—	—	18	18	—	—	4	30	—
20	—	—	—	—	18	18	—	—	4	24	—
21	—	—	—	—	18	18	—	—	4	23	—
22	—	—	—	—	18	18	—	—	4	14	19
23	—	—	—	—	18	18	—	—	3	23	19
24	—	—	—	—	18	18	—	—	7	7	14

* Synovial fluid from knee joints: 0·5 ml. added to each culture.
† Conjunctival swab moistened with 192/2 and placed in 2 ml. of 192/2: 0·5 ml. added to each culture.
‡ Penicillin and streptomycin resistant pleuro-pneumonia-like organism found in passage cultures.

February, 1956, the symptoms following repeated exposures to infection in the previous 2 weeks. When he was examined 55 days later, no urethral discharge remained, but an effusion permitting aspiration was present in the right knee. The addition of this synovial fluid to Hela cultures caused no change in the cells as compared to their controls.

Apart from the systematic studies described, three other single experiments were performed with pooled washings from patients of the Hela cell series of cases. To human kidney cultures in 2 per cent. calf serum medium 0·1 ml. inocula of pools of three and of ten stored washings were added, and a

similar procedure was employed for the pool of ten washings when tested against monkey kidney cultures. At no time did any change suggestive of a viral agent appear during the 15 days' observation. The same pool of ten cases was also used for a "blind passage" experiment with Hela cells in both 2 per cent. calf serum medium and Connaught medium 857 unsupplemented with serum. Three "blind" passages at 9-day intervals were made, the fourth series of cultures being observed for 15 days, but again no cytopathogenic change resulted.

Summary

Urethral washings from 24 cases of non-gonococcal urethritis, and synovial exudate from two patients with Reiter's syndrome, were investigated in Chang conjunctival and amnion cell cultures for the production of viral cytopathogenic effects. Although various non-specific degenerative changes were observed in the cultures, requiring passage for fuller elucidation, no continuously passageable cytopathogenic effect was found, and thus no evidence supporting a viral aetiology in non-gonococcal urethritis or Reiter's syndrome was obtained.

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Addendum

At the time this investigation was performed it was not known that many established cell strains carry PPLO as symbiotic contaminants of the cultures. In Case 9 (Table II) a cytopathogenic effect, passageable seven times at 1/10 dilutions, was attributed to PPLO because these were found in the affected cells, but subsequently the conjunctival strain has been shown to be contaminated with PPLO. The cytopathogenic effect is therefore now being re-examined to identify the responsible agent. The PPLO contamination of the conjunctival strain may have been a cause of the inconsistent behaviour of the strain during the investigation.